per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Identity.* (i) To a solution of 2 milligrams of the sample in 5.0 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine. Boil for 1 minute and cool. A blue color is a positive test.

(ii) To a solution of 2 milligrams of the sample in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10) and mix well. Add, dropwise, 5 drops of a cupric sulfate solution (1:100), mixing after the addition of each drop. A reddish-violet color is a positive test.

[40 FR 22253, May 22, 1975, as amended at 50 FR 19920, May 13, 1985]

§ 448.30a Sterile polymyxin B sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its residue on ignition is not more than 5 percent.

(viii) If it is intended for systemic medication, its heavy metals content is not more than 100 parts per million.

(ix) It gives positive color identity tests for polymyxin.

(2) *Labeling.* In addition to the requirements of § 432.5 of this chapter, if the drug is packaged for dispensing its labeling shall bear the statement, ''Caution: This drug should be given intramuscularly and/or intrathecally only to hospitalized patients so as to provide constant supervision by a physician''.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, heavy metals, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the drug is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers plus one additional package containing 1 gram of the batch.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 2 milliliters of sterile distilled water for each 5 milligrams of weighed sample. Further dilute an aliquot of this solution with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion

from each container. Dilute with sufficient solution 6 to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20,000 units of polymyxin B per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(9) *Identity.* (i) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops; and

(ii) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of cupric sulfate solution (1:100), mixing after the addition of each drop; a reddish-violet color is produced.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16683, Mar. 13, 1981; 46 FR 22359, Apr. 17, 1981; 50 FR 19920, May 13, 1985]

§ 448.75 Tyrothricin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tyrothricin is a white to brownish-white compound of a kind of tyrothricin or a mixture of two or more such compounds. It consists principally of gramicidin and tyrocidine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,400 micrograms of tyrothricin per milligram.

(ii) Its loss on drying is not more than 5 percent.

(iii) It gives a positive identity test for tyrothricin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, and identity.

(ii) Samples required: five packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 95 percent ethyl alcohol, U.S.P. XVIII or equivalent, to give a stock solution of convenient concentration. Further dilute the stock solution with 95 percent ethyl alcohol, U.S.P. XVIII or equivalent, to the reference concentration of 0.20 microgram of tyrothricin per milliliter (estimated). Average the absorbance values for the tyrothricin sample and read the gramicidin concentration from the gramicidin standard response line. Multiply by 5 to obtain the number of micrograms of tyrothricin in the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Identity.* To 5 milliliters of *p*-dimethylaminobenzaldehyde (T.S.) add about 5 milligrams of tyrothricin. Shake well for 2 minutes; then add 2 drops of 0.1*M* sodium nitrite and 5 milliliters of water. A blue color is produced.

Subpart B—Oral Dosage Forms

§ 448.121 Colistin sulfate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for oral suspension is a dry mixture of colistin sulfate, with or without one or more suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, and flavorings. The colistin sulfate content is 5.0 milligrams of colistin per milliliter of the reconstituted suspension. Its potency